

RECIPROCAL CHORIO-ALLANTOIC TRANSPLANTS OF EMBRYONIC DUCK AND CHICK KIDNEY TISSUE

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The results of reciprocal heteroplastic transplantations are not always the same. Such observations have appeared sporadically in the literature, particularly pertaining to grafting experiments between different species of amphibians. Similar observations on warm-blooded vertebrates are not as common. Loeb (1920) grafted kidney tissue between various forms. Some of these grafts were made reciprocally, insofar as host-donor relationships were concerned, between pigeon and guinea pig, and rabbit and guinea pig. More recently, Loeb and King (1935), and Loeb (1935*a* and 1935*b*) made a number of reciprocal transplantations which included such combinations as Gray Norway rats and mutant races, rat and mouse, rat and guinea pig, cat and rat, and rat and pigeon. Adult forms were used in these experiments, which involved transplants of thyroid, cartilage, ovary, and closely associated tissues. In general, the results showed that the grafts between the more closely related forms were more favorable than those between the distantly related forms. In the former there was some indication that a parallelism existed between the intensity of the reaction and the genetic relationship of the host and donor. In the latter, it was difficult to demonstrate this parallelism because necrosis appeared so rapidly in the transplanted tissue that it interfered with the more significant reactions which appear against living tissue. Under these conditions the results of the reciprocal transplants were, for the most part, similar, but some differences were noted.

Sandstrom (1932, 1934, and 1935), although not concerned with reciprocal transplants, reported a series of experiments designed to study the problem of heteroplastic grafting. His experiments were limited to that period in embryonic development when both the host and donor developed those properties responsible for the incompatibility displayed in adult tissue grafts. His results demonstrated that, in addition to the species specificity of the tissues, a number of inter-related factors must be considered in interpreting the results, e.g., the degree of differentiation of the donor tissue, the size of the transplant, the rate of incorporation of the transplant by the host, the capacity of

the transplant to function in its new environment, and the possibility that the grafted tissue might become adapted to the host tissues.

The previous experiments (1932, 1934, and 1935) having been concerned with duck kidney tissue transplants to the chorio-allantoic membrane of the chick, the question arose as to whether grafts of the reverse host-donor relationship (i.e., metanephric tissue of the chick embryo grown on the chorio-allantoic membrane of the duck) would give similar results, and if not, whether a comparison of the reciprocal grafts would give any additional information pertaining to the general problem of heteroplastic transplantations.

A preliminary report on experiments, made with this thought in mind, has already been presented in abstract form (Sandstrom, 1933). During the interim additional transplants were made to fill gaps in the experimental series, and to corroborate earlier observations.

MATERIAL AND METHODS

The procedure used in these experiments was similar to that described by Sandstrom (1934). Briefly, it consisted of implanting a small bit of donor tissue on the chorio-allantoic membrane of the host where it was allowed to grow for the desired length of time, within the limits prescribed by this method of experimentation. In the present set of experiments, however, reciprocal transplants were made between duck and chick embryos, i.e., duck tissue was transplanted to the chick membrane, and chick tissue was transplanted to the duck membrane. The metanephros was again used as a source for donor material because it is an actively functional tissue (in contrast to cartilage, for example, which is considered passively functional), and, under proper incubation conditions, is known to differentiate in either homoplastic or heteroplastic chorio-allantoic grafts (Sandstrom, 1932). The donors were White Pekin duck embryos of 13, 16, and 24 days incubation, and single-comb White Leghorn chick embryos of 9, 13, and 19 days incubation. The hosts were White Pekin duck embryos and single-comb White Leghorn chick embryos of 14 and 9 days incubation, respectively.

Since the degree of differentiation is an important factor in determining the capacity of tissue to grow on the chorio-allantoic membrane, it was necessary, in order to make valid comparisons between the reciprocal transplants, to select hosts and donors of approximately the same stage in development. This is, of course, a problem in itself. The question immediately arose as to whether the chick, which hatches on the twenty-first day of incubation, is in the same stage of development at the time of hatching as is the duckling which hatches on the

twenty-eighth day of incubation. Or is the latter more developed because of the additional week in the shell, and therefore equivalent to the one-week old chick? Either a gross, or histological study of the embryonic structures, such as is used in determining the degree of development in the earlier somite stages of avian embryology, for example, is of little value after visible differentiation has ceased. Since the metanephros was used again as donor tissue, and for the want of a better method in determining equivalent development, it was assumed that the changes occurring in the physico-chemical nature of the epithelial cells of the proximal convoluted tubules, as indicated by the manner in which they stored vital stains, would approximate the desired criterion. Hurd (1928) showed that trypan blue was stored in the form of bright granules in the metanephros of the chick embryo of 19 days incubation. Using the same kind of dye, Sandstrom (1935) determined that it was stored as well-defined masses in the kidney of the duck embryo of 24 days incubation. Assuming that these stages, therefore, are of equivalent development, from the ratio thus established, it was inferred that the chick and duck were approximately equivalent at the time of hatching. On this basis, insofar as it was practical, the ages of the hosts and donors used in the reciprocal transplantations of these experiments were selected.

The grafts were removed at 24-hour intervals after an initial period of 48 hours. They were fixed in Zenker's formal, sectioned at 6 and 8 micra, and stained with methylene blue-eosin. The present report is based on the study of 233 grafts.

COMPARISON OF GRAFTS

Series I—Chick and Duck Donors of 9 and 13 Days Incubation, Respectively

At the time of transplantation, the metanephric tissue of the donors, whether chick or duck, possessed scattered tubules, and sometimes occasional renal corpuscles. It was still able to continue its differentiation when implanted on the chorio-allantoic membrane of the host, for considerable metanephrogenous tissue was still present. The actual condition of the transplanted tissue, however, varied according to the length of time the grafts were allowed to grow.

A comparison of the grafts of metanephric tissue of the chick embryo grown on the chorio-allantoic membrane of the duck for 2, 3, and 4 days, with those of the duck grown on the chick membrane for identical periods, showed similar conditions. All of the grafts were well incorporated. Differentiation had not progressed to any great extent in those removed on the second day; it appeared much as it did

at the time of transplantation. In the grafts removed on the third and fourth days, the amount of metanephrogenous tissue was considerably reduced, and the transplants consisted largely of uriniferous tubules which, in some instances, could be differentiated into their various parts. But the tissue in the grafts removed up to this time was not all normal, particularly in those taken on the second day. Centrally, it appeared as though in the initial stages of necrosis. Instead of taking the methylene blue stain as did the more peripheral normal tissue, it stained with eosin only. The cytoplasm of the cells of the tubules contained vacuoles, and the nuclei were shrunken. This condition was undoubtedly due to the inability of the blood vessels to penetrate into the transplant rapidly enough to insure proper vascularization. Accompanying this apparently necrotic condition were numerous small lymphocytes. By the third and fourth days there was considerably more normal, and less necrotic, tissue. At the same time there occurred a proportional reduction in the number of small lymphocytes. They were usually limited to the small areas of tissue in which necrosis had definitely set in.

Upon comparing the reciprocal grafts removed on the fifth and sixth days, one prominent difference was noted which was not apparent in any of the transplants removed earlier. The incorporation of the metanephric tissue of the chick into the chorio-allantoic membrane of the duck was less complete than that of the duck into the chorio-allantoic membrane of the chick. The failure of the chick tissue to become completely incorporated manifested itself in two ways. In some instances, the grafts were taken into the host membrane, but only a small part of the surface of the transplant came in contact with the host tissue, and the region where the loose connective tissue and blood vessels of the chorio-allantoic membrane could enter was therefore limited considerably. Usually this contact was confined to one side. The remaining surface of the graft was separated from the host tissue by a space which was occasionally filled with a colloid-like substance. The tissue was normal.

In other instances, the grafted tissue was not completely incorporated. The impression gained from an examination of the sectioned material was that the chick tissue, although at first fully incorporated into the duck membrane, was being forcibly ejected. The inner side of the transplant was in contact with the host tissue, and the loose connective tissue and blood vessels of the host membrane entered the graft in this region only. The inner part, therefore, was, in a sense, incorporated, and was normal in all respects. But in passing toward the outer part, the tissue became more and more necrotic. The

tubules were crowded, and their shapes altered considerably. It was still possible, nevertheless, to detect that some differentiation had taken place on the membrane. The outer part of the graft was definitely necrotic. It was not covered by the chorionic epithelium, the loose edges of which were found about the periphery, and the dead tissue was sloughing into the space between the chorionic surface and the inner shell membrane.

It is to be understood, however, that incomplete incorporation, such as described above, was not universal among the grafts of chick tissue on the duck membrane. Other grafts, in the minority to be sure, were incorporated fully as well as the duck-on-chick transplants. Regardless of the host-donor relationship, those grafts that were sufficiently incorporated to insure proper vascularization to all regions showed a high degree of differentiation with all parts of the typical kidney present, and little necrosis. Lymphocytes were not common, even in the grafts containing necrotic tissue.

There were more notable differences between the reciprocal grafts that were removed from the seventh to the tenth days. Although most of the tissue in the duck transplants grown on the chick membrane was normal, conditions were quite variable in the grafts of the reverse host-donor relationship. A few small grafts contained normal chick tissue, others showed varying amounts of necrotic tissue, and still others were in the process of sloughing in the manner already described. In some cases the transplanted chick tissue had completely sloughed from the host membrane, and the only evidence of a previous graft was an increased amount of connective tissue in the general region. In addition, there was another striking difference between the two types of transplants. In the majority of grafts of chick tissue removed from the duck membrane during this period, intense infiltrations of granulocytic leucocytes and many small lymphocytes made their appearance. Generally granulocytes are not uncommon in metanephric tissue grafts, but they are usually present in small numbers only. They are normally formed from the interstitial connective tissue of the developing metanephros, and their existence in the transplant is to be expected. But in the chick grafts removed from the duck membrane on, or after, the seventh day, they manifested themselves in a manner not found in any of the duck-on-chick grafts. They were so numerous that the transplant, in some instances, was completely obscured. They were also found in the connective tissue of the chorio-allantoic membrane immediately surrounding the graft. Wherever the granulocytes were sufficiently scattered so that the transplant could be studied adequately, its tissue was found completely incor-

porated and, for the most part, histologically normal. Some necrotic tissue, however, was present. The intensity of the granulocytic infiltrations could not be correlated with the amount of necrosis, for grafts which appeared almost completely normal were as intensely infiltrated as those that contained large necrotic areas.

A study of the chorio-allantois immediately around the grafted tissue revealed the source of the great numbers of granulocytes. It was quite obvious that the presence of the foreign tissue had stimulated the formation of granulopoietic centers, much in the same manner that homoplastic chorio-allantoic grafts of adult chicken spleen stimulated the formation of centers of granulopoiesis as described by Danchakoff (1918). In fact, many of the stages in the differentiation of the granulocytes, as described by her, were found. The loose connective-tissue cells had enlarged to form relatively large amoeboid cells—the hemoblasts. Various stages could be selected to form a sequence of transformations leading from the hemoblast to the granuloblast. The final stage in this selected line of differentiation, the granulocyte, was very common. All of these stages were limited, for the most part, to circumscribed areas. These loci were located at various distances from the grafted tissue. In some cases they were directly adjacent to the transplant so that the various stages in the differentiation of the granulocyte were within the grafted tissue. More often, the loci, while in the immediate vicinity, were slightly removed from the graft, and in these cases, only fully differentiated granulocytes were found in the transplant.

Small lymphocytes, as well as granulocytes, were present, both in the grafted tissue and surrounding host membrane. They were not as common as the granulocytic leucocytes, and were somewhat larger than the small lymphocytes found in the necrotic-appearing areas of grafts removed 2 days after implantation. No evidence was found which would indicate the loci of their formation as coincident with the granulopoietic centers, although small lymphocytes were present in large numbers in these centers. Unlike the results of Danchakoff (*loc. cit.*), no convincing sequence of cell types could be selected that would suggest their transformation from the hemoblasts.

*Series II—Chick and Duck Donors of 13 and 16 Days Incubation,
Respectively*

The reciprocal transplants of this series showed decided differences, although at the time of transplantation, the metanephroi of both donors had reached a comparable degree of histological differentiation. The metanephric tissue of the duck continued its growth and development on the chick membrane in the manner reported on previous occasions

(Sandstrom, 1932 and 1934). Considered briefly, the condition of the transplant varied according to the length of time the graft was allowed to remain on the host membrane. There was an initial period of 2 or 3 days when the final stages of incorporation were in progress, after which the implant was completely taken into the chorio-allantoic membrane and covered by the chorionic epithelium. During this period, the peripheral tissue was apparently normal, whereas the central part showed the early stages of necrosis, both as to its structural appearance and staining reactions. Numerous small lymphocytes were present in, and about, the necrotic area. Grafts removed subsequently, e.g., from the fourth to the tenth days, showed progressively less necrotic, and more normal tissue, as the length of time they grew on the membrane increased, so that ultimately their tissue, for the most part, was comparable to that of a normally developed metanephros of equivalent age. But some necrosis was present even in the grafts removed during the later intervals, the extent varying from small areas to complete grafts. The number of grafts containing necrotic tissue, however, was decidedly in the minority.

Upon comparison, the grafts of the reverse host-donor relationship, i.e., metanephric tissue of the chick grown on the chorio-allantoic membrane of the duck, showed conditions quite in contrast to the duck-on-chick grafts. A most striking difference was apparent in the process of incorporation, which, in most cases, was considerably retarded. Regardless of the length of time the grafts were allowed to remain on the duck membrane, the implanted tissue was rarely completely incorporated. It remained, more or less, in a stage comparable to that found in the duck-on-chick transplants removed from the host membrane within 48 hours after implantation. The epithelial cells of the membrane, undoubtedly stimulated in some manner by the presence of the implanted tissue, increased in number, forming epithelial projections which extended into the implant. Thus, that part of the metanephric tissue which was in immediate contact with the chorionic epithelium became incorporated first; it was "engulfed" by the host epithelium. Peripherally, the chorionic epithelium folded upward about the transplant, much as the somatopleure folds over the developing chick embryo in the formation of the amnion and chorion. Usually these folds extended upward only a short distance. Rarely did they cover the graft completely, and if so, not before the eighth day. In these cases, the inner part of the fold was sometimes found, much folded, in the mesoderm of the membrane, and not infrequently formed a hard, cornified mass.

Obviously, the tissue of the transplant was considerably affected

by the retarded incorporation. That part which was "engulfed" by the epithelium was normal in all respects. It procured sufficient vascularization with little delay, and continued its development, so that the grafts removed from the eighth to the tenth days, for example, contained apparently normal, fully differentiated metanephric tissue. Necrosis appeared rapidly in the remaining unvascularized part, which was usually left outside the membrane, although occasionally it was completely covered by the chorionic epithelium. When covered, the dead tissue was always separated from the normal either by a space, or connective tissue.

*Series III—Chick and Duck Donors of 19 and 24 Days Incubation,
Respectively*

The high degree of differentiation that the donor tissue, whether chick or duck, had attained at this stage of development, was reflected in the incapacity of the transplants to grow on the chorio-allantoic membrane. Regardless of the host-donor relationship, no great amount of normal tissue was found in the grafts. That which was present probably came from small scattered areas of metanephrogenous tissue that were still present at the time of transplantation, or represented tissue which was sometimes found to persist through the period that the grafts remain on the host membrane. Only one graft, a chick-on-duck, was completely normal.

One prominent difference was apparent between the reciprocal grafts of this series. The metanephric tissue of the chick was almost always completely incorporated into the duck membrane, even though it was seldom capable of continuing its growth: This complete incorporation of the implant was in striking contrast to the conditions found in similar grafts of the previous series (Series II), where incomplete incorporation was almost universal regardless of the stage of removal. It was also in striking contrast to the results obtained in the reciprocal grafts, i.e., duck-on-chick, of this series, which resembled, to a degree, the conditions prevalent in some of the chick-on-duck grafts of Series I. During the early period of incorporation, the implanted tissue, completely taken into the chick membrane, consisted of the characteristic peripheral area of normal tissue and the relatively larger central area of apparently necrotic tissue. Numerous small lymphocytes were aggregated in and about the central part. From the fourth day on, however, much of the grafted tissue was found in the process of sloughing. Not infrequently, it had sloughed from the membrane completely, and the area once occupied by the duck tissue was filled with connective tissue.

DISCUSSION

The foregoing comparison of the embryonic duck and chick transplants demonstrated that a definite reciprocal relation existed between the host and donor species only during early embryonic development. During this period the metanephrogenous tissue of both the duck and chick were equally well incorporated into the chorio-allantoic membrane of their respective hosts. As development proceeded, however, this reciprocal relation was, to a considerable extent, lost. Pronounced differences appeared in the transplants. For example, the implanted metanephric tissue of the 14-day chick embryo was incorporated less completely, and more slowly, than the equivalently developed tissue of the 19-day duck embryo was into the chick membrane. This retarded incorporation is demonstrated, not only in many of the grafts of Series I (donors of 9 days incubation) that were removed from the host membrane on, and after, the fifth day, but somewhat more decisively in most of the grafts of Series II (donors of 13 days incubation), irrespective of the time of removal.

Other differences were noted. In Series I, completely incorporated chick tissue, after having grown in the new environment from 7 to 10 days, often stimulated the formation of leucopoietic centers in the neighboring regions of the duck membrane. No stimulation of this kind resulted from the transplantation of duck tissue to the chick. Furthermore, in Series III, the implants of fully differentiated, functional metanephric tissue from both the chick and duck (ages of 19 and 24 days incubation, respectively) were completely incorporated, with no obvious delay, into the membranes of their respective hosts. In this group, however, it was the transplanted chick tissue that grew, or at least persisted, in its new environment, whereas the grafted duck tissue, after having grown for a few days, frequently sloughed from the membrane without creating any apparent disturbance in the neighboring host tissue. And finally, closely correlated with the retarded rate of incorporation, considerably more necrotic tissue was found, in general, in the grafts of chick tissue than in those of duck tissue.

Undoubtedly, the reactions described in the foregoing paragraph were associated with the development of species specificity in the donor tissues. Its appearance in the metanephric tissue of the chick obviously occurred on the fourteenth day of incubation, the time when the incorporation of the chick tissue was first retarded. But as for the appearance of species specificity in the duck tissue, no actual evidence is available from these experiments. Assuming that the method used in determining equivalent development is essentially

correct, it should be present at approximately the nineteenth day of incubation. As a matter of fact, this conclusion checks with that of Sandstrom (1934) who, from a study of heteroplastic and homoplastic chorio-allantoic grafts of duck kidney tissue, experimentally demonstrated its presence at this time.

Although it is essential that the transplanted metanephric tissue should function in order to continue its growth on the chorio-allantoic membrane, as was shown by Sandstrom (1934 and 1935), apparently its incapacity to do so does not elicit a reaction from the host. At least this was true for the duck-on-chick transplants. More than likely, therefore, the lack of function in the chick-on-duck transplants cannot be considered an important factor in causing the reactions found in these grafts. That the metanephros of the chick has developed function by the fourteenth day of incubation is suggested by the results of Gersh (personal communication). By a refined technique, he has been able to demonstrate activity in the tubules of the chick metanephros as soon as they are structurally differentiated, i.e., starting the eleventh day. According to his observations, however, only a very few tubules are sufficiently differentiated at this time, and the number increases but slowly, making it highly improbable that the incapacity to function played an important part in instigating the reaction obtained.

Since the species specificity of the transplanted tissue was the primary factor responsible for the reactions between graft and host, the differences in these reactions, as found upon comparison of the reciprocal grafts, must be ascribable to differences in the capacity of this factor in the donor tissues to instigate reactions in the membranes of their respective hosts. The more determined reactions obtained from the chick implants, as compared with the duck, suggest that either the species specificity factor of the former is the more potent of the two, or the chorio-allantoic membrane of the duck is the more reactive. In either case, it would be the donor that provoked the reaction, the character of which would necessarily have to be a function of the host. The reactions obtained in the reciprocal grafts, therefore, differed. These results are well in accord with the statement made by Loeb (1930, p. 572) that "if the relation between host and donor is reversed, the results of transplantation, as manifested in the intensity of the reaction against the transplant, do not need to be identical; no simple reciprocity therefore exists. This can be readily understood, if we consider that it is the host which reacts against the transplant, and not the transplant which reacts against the host, or at least that, under ordinary circumstances, we have no means of determining the reaction on the part of the transplant against the host."

Incidentally, it is difficult to understand how the donor tissue, without reacting against the host, can provoke a reaction in the host, as implied in the statement by Loeb, quoted in the previous paragraph. As a matter of fact, an analysis of the process of incorporation, as it occurred in the chorio-allantoic grafts of chick tissue on the duck, suggests that the donor does react against the host. Although chorio-allantoic grafting as a method for study of heteroplastic transplantation may possess certain shortcomings, it has the advantage over other methods in that the incorporation of the implant can be used as an index of reactivity. The implant is carefully placed on the surface of the chorionic epithelium, so that the epithelial tissue is involved before the foreign transplant finally becomes lodged in the connective tissue of the membrane. No actual mechanical injury, therefore, is incurred by the host. This method is in contrast to that of subcutaneous transplantation, for example, as practiced with adult animals, in which the implant is placed directly into the subcutaneous connective tissue, thereby mechanically assisting the process of incorporation. In addition, since mechanical injuries to the recipient's tissues are unavoidable results of this procedure, the incorporation of the implanted tissue cannot be used in the study of either host or donor reactions. In the chorio-allantoic grafts under consideration, therefore, the process by which the implant was incorporated assumed considerable importance. The only connection between the host and donor tissues at the time of implantation was a physical contact between the surfaces of the implant and chorionic epithelium, and, it will be recalled from the description of the results, that the epithelium subsequently "engulfed" portions of the implanted tissue. Under these conditions, the rate of incorporation was determined by two opposing factors—an apparently natural tendency for the epithelium to react favorably toward the implant, as indicated by the manner in which it "engulfed" parts of the implanted tissue, and the presence of the species specificity factor in the donor tissue which inhibited the "engulfing" activity of the host. The amount of tissue incorporated was apparently dependent on the relative effectiveness of these two factors. In any event, the analysis has indicated that some of the responsibility for the retarded incorporation rests with the reaction of the transplant against the host.

It has been emphasized that the species specificity factor of the donor tissue was of primary importance in causing the reactions in the transplants; therefore further consideration must be given to the seemingly contradictory results obtained in the grafts of Series III, in which fully differentiated, species specific tissue was not only readily

incorporated, but even failed to call forth a reaction from the host. From the results of the chick-on-duck transplants it was evident that, insofar as its capacity for eliciting a host reaction is concerned, the developing donor tissue passed through three stages: during the first, which obtained previous to the development of species specificity, the host and donor tissues were compatible; the second obtained during the development of species-specific properties which caused a definite reaction; and the third obtained subsequent to the appearance of species specificity when no reaction occurred. Owing to the milder response, these stages, although present, were not very apparent in the duck-on-chick grafts.

Such an unusual sequence is not unparalleled among grafting experiments, for an analogous situation was found existent in some of the results mentioned by Loeb (1930) in a review of his theory of organismal differentials. This author, referring particularly to the intensity of the reaction which appeared against his transplants of non-resistant tissues (e.g., thyroid, made between variously related adults), as indicated by the lymphocytic infiltrations, stated that few, or no, lymphocytes were found in autoplasmic transplants. According to Loeb's conception, the lymphocytic reaction is a response toward a toxin produced by the transplant. Inasmuch as host and donor were the same individual, and had identical individuality differentials (chemical characteristics), no toxic substance was produced to attract the lymphocytes. In homoplasmic transplants, although the respective individuality differentials of the host and donor differed, this difference was not great enough to interfere with the metabolic activity of the transplant. Sufficient toxin was produced, therefore, to call forth a decided infiltration of lymphocytes. In heteroplasmic transplants, the individuality differentials of the host and donor were so different that the body fluids of the former, acting directly on the latter, caused degenerative changes therein. These changes interfered so extensively with the metabolism of the implant that only a slight amount of toxin was formed. As a result, the intensity of the lymphocytic reaction was less pronounced, and appeared more slowly, than that which appeared against the homoplasmic transplants.

Insofar as the intensity of the reaction is concerned, therefore, the sequence obtained in the chorio-allantoic grafts of developing metanephric tissue not only seems to recapitulate the conditions described in the preceding paragraph, but the explanation postulated by Loeb, in consideration of his results, may also be applicable. The conditions found in the chick-on-duck grafts of the first stage are comparable to those found in the autoplasmic transplants; no reactions occurred. In

the grafts of the second stage, however, the intensity of the reactions resembled that of the lymphocytes in the homoplastic transplants. The individuality differential of the donor had developed sufficiently to excite a reaction without interfering appreciably with its capacity to live in the new environment. Furthermore, the implanted tissue, because it was still undifferentiated enough to be plastic, could be handled, etc., during the operative procedures without causing considerable degenerative changes. Thus this metabolically active tissue, perhaps through the agency of a toxin, affected the host tissues in a decisive manner. In the final stage of the sequence, e.g., grafts of Series III, true heteroplastic conditions were being approximated; the development of the individuality differential (species specificity factor) had reached completion. Even though the differentials of the host and donor resembled one another less than in the previous stage, the host was unresponsive toward the implant during the limited time it remained on the membrane. As in the case of heteroplastic transplants of Loeb, the intensity of the reaction was undoubtedly diminished because of degenerative changes that occurred in the implant. To what extent the body fluids present in the chorio-allantoic membrane of the unhatched host were capable of causing these changes is doubtful. It is more than likely that the changes were caused by injuries incurred during the operative procedure, since the plasticity of the tissue had decreased considerably. In addition, vascularization, so essential to a differentiated, functional tissue, was necessarily retarded because the process of incorporation, as it occurs in chorio-allantoic transplants, at best, is relatively slow. Under such conditions the implant could not have consisted of an actively metabolizing tissue, and thus lacked the essential prerequisite for calling forth a reaction.

SUMMARY

1. Metanephric tissue from duck and chick embryos that were approximately equivalent in development was transplanted to the chorio-allantoic membrane of the chick and duck respectively. The hosts, also, were in comparable stages of development.

2. A reciprocal relation was found to exist during the early embryonic stages only. As development progressed this relation was lost, to a large extent; prominent differences occurred in the two types of transplants. Whereas relatively slight or no reactions were observed in the duck-on-chick grafts, intense reactions appeared in the grafts of the reverse host-donor relationship.

3. The reactions appearing in the grafts were associated with the development of the species specificity factor in the donor tissue.

Its appearance in the chick tissue occurred at about the fourteenth day of incubation.

4. The reactions which appeared as a result of the development of the species specificity factor were: (1) a decided retardation of the rate of incorporation of the implanted tissue; (2) possible expulsion of the transplant from the membrane; and (3) the stimulation of leucopoietic centers in the connective tissue of the surrounding host membrane. These reactions, if present in the duck-on-chick grafts, were scarcely observable because of their mildness.

5. It was demonstrated by these experiments that, although the host reacted against the transplant, which obviously instigated the reaction, it was also possible, as was shown by an analysis of the process of incorporation, for the transplant to react against the host.

6. Seemingly contradictory results were obtained in the grafts of fully differentiated, species specific tissue, in that the implants were readily incorporated into the host membrane, without provoking a reaction. Although no evidence was offered, an explanation of these unusual results was suggested which was based on the theory of Loeb (1930) that the reaction against a transplant is dependent, to a large measure, on the active metabolism of the transplanted tissue.

LITERATURE CITED

- DANCHAKOFF, V., 1918. Equivalence of different hæmatopoietic anlagen. II. Grafts of adult spleen on the allantois and response of the allantoic tissues. *Am. Jour. Anat.*, **24**: 127.
- HURD, M. C., 1928. Observations on the storage of trypan blue in the embryo chick. *Am. Jour. Anat.*, **42**: 155.
- LOEB, L., 1920. Heteroplastic transplantation of kidney. *Jour. Med. Research*, **42**: 137.
- LOEB, L., 1930. Transplantation and individuality. *Physiol. Reviews*, **10**: 547.
- LOEB, L., 1935a. Transplantation of tissues from mouse to rat and vice versa. *Am. Nat.*, **69**: 239.
- LOEB, L., 1935b. Comparison of the reactions against heterotransplanted tissues in different kinds of hosts. *Biol. Bull.*, **68**: 440.
- LOEB, L., AND H. D. KING, 1935. The analysis of the organismal differentials of Gray Norway rats and of two mutant races by means of transplantation. *Am. Nat.*, **69**: 5.
- SANDSTROM, C. J., 1932. The growth and differentiation of duck kidney tissue on the chorio-allantoic membrane of the chick and duck. *Physiol. Zool.*, **5**: 354.
- SANDSTROM, C. J., 1933. Transplantation of chick metanephric tissue to the chorio-allantoic membrane of the duck (abstract). *Anat. Rec.*, **57**: 80.
- SANDSTROM, C. J., 1934. Additional observations on heteroplastic chorio-allantoic grafts of embryonic duck kidney tissue. *Physiol. Zool.*, **7**: 279.
- SANDSTROM, C. J., 1935. The storage of trypan blue in heteroplastic chorio-allantoic grafts of embryonic duck kidney tissue. *Anat. Rec.*, **62**: 7.